RESEARCH PAPER

" MULTI-TARGET MECHANISTIC INSIGHTS INTO GARLIC-MEDIATED NEUROPROTECTION IN ALZHEIMER'S DISEASE USING NETWORK PHARMACOLOGY, MOLECULAR DOCKING, AND IN VITRO VALIDATION "

Bhumika Gurve[1], Tabbasum Tamboli[1], Shubham Nalawade[1], Vikram More[1], Aniket Aute[1]*
[1] Adarsh College of Pharmacy, Vita, Maharashtra, India
Corresponding Author**:** aniketaute1121@gmail.com

ABSTRACT:-
Molecular docking, in vitro cell line study, network pharmacology, and molecular dynamics (MD) simulations are all used to assess Allium sativum's anti-Alzheimer potential in this research. The molecular pathways linked to Alzheimer's disease (AD) remain poorly understood, despite the widespread use of Allium sativum in traditional medicine for its antioxidant and neuroprotective effects. Allicin, alliin, ajoene, diallyl sulfide, and S-allyl cysteine were among the main bioactive components found during phytochemical screening. Some common targets linked to Alzheimer's disease were identified by target prediction and disease-gene mapping studies based on these phytochemicals. Several biochemical pathways related to cholinergic neurotransmission may be regulated by Allium sativum, according to protein-protein interaction (PPI) and enrichment studies, oxidative stress, neuroinflammation, amyloid-beta metabolism, and tau protein regulation.
"The molecular docking studies showed that certain phytoconstituents had much stronger binding affinities than the standard therapeutic agents for several important Alzheimer's-related targets, such as acetylcholinesterase (AChE), butyrylcholinesterase (BChE), amyloid precursor protein (APP), and microtubule-associated protein tau (MAPT). In addition, the MD simulations showed that the protein-ligand interactions were stable and that the conformational dynamics were favorable, which might have biological implications and modulate the target. An in vitro acetylcholinesterase inhibition study confirmed the neuroprotective potential of garlic extract, complementing the computational findings. The extract showed substantial enzyme inhibitory activity that was on par with that of conventional inhibitors. In conclusion, this study shows that Allium sativum has the ability to modulate important proteins and signaling pathways linked to cognitive impairment, neurodegeneration, inflammation, and oxidative stress, suggesting that it could be a natural treatment option for Alzheimer's disease.
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1. INTRODUCTION

Symptoms of Alzheimer's disease (AD) include a gradual deterioration in cognitive abilities, loss of memory, and trouble with day-to-day functioning. It places a heavy strain on healthcare systems, especially in populations that are becoming older, and is the leading cause of dementia globally. The development of Alzheimer's disease (AD) is a multi-step process that begins with the buildup of amyloid-beta (Aβ) plaque and progresses via many pathways such as tau protein hyperphosphorylation, neuroinflammation, oxidative stress, neuronal destruction, and cognitive impairment [1].
Memantine, an antagonist of the N-methyl-D-aspartate (NMDA) receptor, and acetylcholinesterase inhibitors like donepezil, rivastigmine, and galantamine are the mainstays of current therapy methods. Nevertheless, these remedies just alleviate symptoms and are often linked to ineffectiveness and negative side effects [2]. So, finding multi-target therapy drugs that can affect the many different biochemical pathways that lead to AD is becoming more important.
Garlic, or Allium sativum L., is a popular medical herb due to its many beneficial effects on health, including reducing inflammation, killing germs, and protecting nerve cells. Its pharmacological effects are mainly due to bioactive molecules that contain sulfur, including allicin, alliin, ajoene, diallyl sulfide, and S-allyl cysteine. These phytoconstituents have been shown in earlier research to have anti-degenerative properties, including lowering oxidative stress and neuroinflammatory reactions. Molecular processes underpinning Allium sativum's anti-Alzheimer potential are still poorly understood, despite these encouraging discoveries [3].
To better understand the interplay between bioactive chemicals, molecular targets, and disease-related pathways, network pharmacology offers a systems-level perspective. Herbal remedies, which often have more than one active ingredient and many targets, lend themselves well to this approach. The binding affinity of phytoconstituents to target proteins may be

predicted further using molecular docking, and the stability and conformational behavior of protein-ligand complexes can be assessed through molecular dynamics (MD) simulations [4]. A complete method for understanding treatment processes may be achieved by combining computational techniques with experimental validation.

This research seeks to examine the therapeutic potential of Allium sativum against Alzheimer's disease by integrating network pharmacology, molecular docking, molecular dynamics modeling, and in vitro validation (fig. 2). Find important bioactive chemicals, figure out where they'll have an effect, investigate the signaling pathways that go along with them, and see how they interact with the main proteins involved in Alzheimer's disease [5]. This comprehensive method sheds light on garlic's multi-target processes and lends credence to its promise as a natural treatment for Alzheimer's disease.

2. MATERIALS AND METHODS

2.1 Plant Material Collection and Authentication

The garlic bulbs, scientifically known as Allium sativum L., are members of the Amaryllidaceae family and were sourced from a nearby farm. For reference purposes, a voucher specimen was preserved and the gathered plant material was validated by a trained botanist from an established institution. On December 5, 2025, the authentication process was finished. We used distilled water to wash the plant material and eliminate any contaminants. After that, it was let to dry at room temperature in the shade. Finally, we used a mechanical grinder to ground it into a fine powder. Until it was needed again, the powdered substance was kept in sealed containers[6].

2.2 Chemicals and Reagents

All chemicals and reagents used in the present investigation were of analytical grade. Distilled water was prepared in-house and used throughout the experimental procedures. Ethanol and methanol employed for extraction were procured from Loba Chemie. Acetylcholinesterase enzyme was obtained from a certified research laboratory. Acetylthiocholine iodide and 5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB; Ellman's reagent) were bought from Sigma-Aldrich [7] for the acetylcholinesterase inhibition experiment. We used established laboratory procedures to make our own phosphate buffer (pH 7.4). In order to compare the anti-Alzheimer activity of different drugs, donepezil was utilized as the reference standard.

2.3 Equipment

The experimental work was carried out using standard laboratory equipment, including an analytical balance, mechanical grinder, hot air oven, UV–Visible spectrophotometer, micropipettes, centrifuge, vortex mixer, water bath, and glassware. Computational studies involving network pharmacology, employing bioinformatics tools and publically accessible datasets, we ran molecular dynamics simulations and molecular docking (Table 1) [8].

Table 1 Software Tools and Online Platforms Used for Network Pharmacology, Docking, and Simulation Studies.

Software/Tool	Application Area	Access Type
SwissADME	ADME prediction	Online (free)
SwissTargetPrediction	Target prediction	Online (free)
GeneCards	Alzheimer's gene identification	Online (free)
STRING	Protein-protein interaction	Online (free)
Cytoscape	Network visualization	Desktop (free)
ShinyGO	Pathway enrichment	Online (free)
RCSB PDB	Protein structure retrieval	Online (free)
CB-Dock2	Molecular docking	Online (free)
Discovery Studio Visualizer	Docking visualization	Desktop (free)
iMODS	Molecular dynamics simulation	Online (free)
IMPPAT	Phytochemical database	Online (free)
DAVID	Functional annotation	Online (free)
MS Excel / Word	Data analysis & documentation	Licensed

Table 2 Predicted cavity details MAOA for protein (PDB ID: 2Z5X)

CurPocket ID	Cavity Volume (Å³)	Center (x, y, z)	Cavity Size (x, y, z)
C1	4358	32,32,-17	30,16,13
C2	1824	45,12,-13	25,13,13
C3	833	49,22,-26	14,13,17
C4	215	25,18,-15	6,11,5
C5	200	43,48,-22	7,6,13

Figure 1. Predicted binding cavities of MAOA (PDB ID: 2Z5X) and docking pose of Allixin

Figure 2. Workflow overview

Figure 3. Venn Diagram

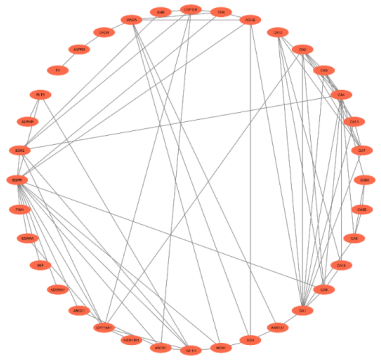


Figure 4. PPI Network of 40 Common Target Genes.

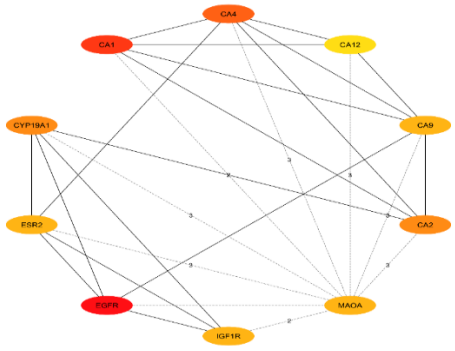


Figure 5. Top 10 Hub Genes Identified Using Degree Method (CytoHubba Visualization)

2.4. Collection Of Alzheimer's Disease-Related Genes

Disease-associated genes for Alzheimer's disease (AD) were obtained from the GeneCards, OMIM, and DisGeNET databases using keyword-based

retrieval. Redundant, duplicate, and unannotated entries were removed, and gene nomenclature consistency was verified using UniProt mapping. Only Alzheimer's disease-associated genes with a GeneCards relevance score ≥10 were included to eliminate low-confidence entries and minimize potential bias arising from weak literature associations [9]. The collected gene dataset was subsequently used for target intersection and network analysis.

2.5. Screening of common targets of Allium sativum and Alzheimer's disease

The intersection between phytoconstituent-related targets of Allium sativum and Alzheimer's disease-associated genes was determined using Venny 2.1.0 (fig. 3). These overlapping targets were considered potential therapeutic mediators of Allium sativum in the treatment of Alzheimer's disease and were selected for subsequent protein–protein interaction (PPI) network construction and enrichment analysis (fig. 4) [10].

2.6. Development of PPI network and identification of central targets

To characterize the interaction landscape of the overlapping genes, a PPI network was constructed using the STRING database with a minimum interaction confidence score of 0.7. The interaction data were exported and analyzed using Cytoscape 3.9.1. Topological properties of the network were evaluated using the CytoHubba plugin to identify key hub genes based on the degree centrality algorithm (Table 3). Topological analysis was further performed using the Network Analyzer plugin in Cytoscape. Parameters including degree centrality, betweenness centrality, and closeness centrality were calculated to identify nodes with the highest regulatory influence within the network (fig. 5) [11]. The identified hub genes were considered potential key targets involved in the therapeutic effects of Allium sativum against Alzheimer's disease.

2.7. Functional annotation through Gene Ontology and pathway mapping via KEGG

Functional annotation of the intersecting genes was performed using the ShinyGO platform, incorporating Gene Ontology (GO) enrichment under the categories of Biological Process (BP), Molecular Function (MF), and Cellular Component (CC) [12]. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was also conducted to identify significant biological pathways associated with Alzheimer's disease (fig 12). Visualization of enriched pathways and functional terms was performed using bar plots, network diagrams, and hierarchical clustering layouts (fig 6-9).

2.8. Construction of phytoconstituent–target–disease network

A multilayered network comprising Allium sativum, its bioactive phytoconstituents, predicted target proteins, and Alzheimer's disease was constructed using Cytoscape. Connectivity analysis was performed using the Network Analyzer plugin to identify key phytoconstituents with high degree values and centrality scores. These highly connected compounds were considered potential active constituents responsible for the neuroprotective effects of Allium sativum in Alzheimer's disease [13].

2.9. Molecular docking and molecular dynamics simulation

Protein–ligand interactions were assessed through molecular docking using CB-Dock2, which integrates cavity prediction and blind docking algorithms [14]. Three-dimensional crystal structures of selected Alzheimer's disease-associated target proteins were retrieved from the RCSB Protein Data Bank (PDB), while the chemical structures of Allium sativum phytoconstituents were obtained from the PubChem database [15-16]. Binding affinity scores generated by the AutoDock Vina scoring function were used as the primary criterion for selecting promising protein–ligand complexes [17]. Interaction profiles and binding modes were visualized in both two-dimensional (2D) and three-dimensional (3D) formats using BIOVIA Discovery Studio.

To ensure structural reliability and improve docking accuracy, all selected hub proteins underwent a comprehensive validation and optimization workflow. Initially, the crystal structures were refined using the PDB-REDO server to improve geometric quality and crystallographic parameters [18]. Subsequently, the refined models were evaluated using the SAVES v6.0 validation suite, which assesses Ramachandran plot statistics, rotamer quality, atomic packing, and hydrogen-bonding patterns [19]. Further structural validation was performed using the ProSA-web server through Z-score analysis to confirm overall model quality and compatibility with experimentally determined protein structures [20]. A total of ten hub proteins identified from the protein–protein interaction (PPI) network were selected for docking studies based on degree centrality values obtained through CytoHubba analysis [21]. These proteins are associated with critical biological processes involved in Alzheimer's disease pathology, including neuronal signaling, neurotransmitter metabolism, hormonal regulation, cellular homeostasis, and neurodegeneration. The prepared protein structures were subjected to docking with major phytoconstituents of Allium sativum, including allicin, alliin, ajoene, diallyl sulfide, and S-allyl cysteine [22].

Docking simulations were performed in CB-Dock2 using the AutoDock Vina scoring function with an exhaustiveness value of 8. The binding cavities of each target protein were predicted automatically by the CB-Dock2 server using a curvature-based pocket detection algorithm, which identifies potential ligand-binding sites and ranks them according to cavity volume. For each protein, multiple binding cavities were generated, and the cavity with the highest volume and optimal geometric characteristics was selected for docking. The corresponding grid center coordinates and cavity dimensions were automatically assigned by the server. Prior to docking, both receptor and ligand structures were energy minimized to eliminate steric clashes and obtain stable conformations.

The resulting docked complexes were ranked according to their binding affinity scores, and the most favorable protein–ligand interactions were selected for further investigation. Hydrogen bonds, hydrophobic interactions, van der Waals forces, and other non-covalent interactions contributing to complex stability were analyzed using BIOVIA Discovery Studio [23].

Table 3 Top ten genes identified from the PPI network using CytoHubba (Degree method)

Rank	Gene Symbol	Degree Score	Full Gene Name
1	EGFR	14	Epidermal Growth Factor Receptor
2	CA1	10	Carbonic Anhydrase 1
3	CA4	9	Carbonic Anhydrase 4
4	CYP19A1	7	Cytochrome P450 Family 19 Subfamily A Member 1
4	CA2	7	Carbonic Anhydrase 2
6	MAOA	6	Monoamine Oxidase A
6	ESR2	6	Estrogen Receptor 2
6	CA9	6	Carbonic Anhydrase 9
6	IGF1R	6	Insulin-Like Growth Factor 1 Receptor
10	CA12	5	Carbonic Anhydrase 12

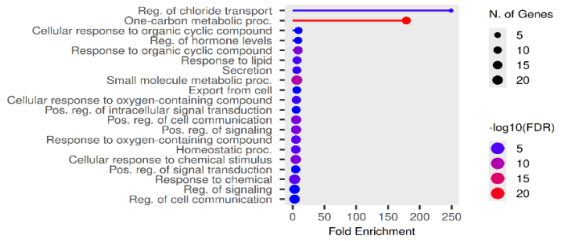


Figure 6. Bar Plot Showing Top Enriched Biological Process Terms Among The 40 Genes.

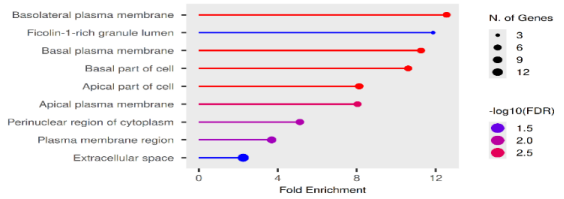


Figure 7. Bar Plot Showing Top Enriched Cellular Component Terms.

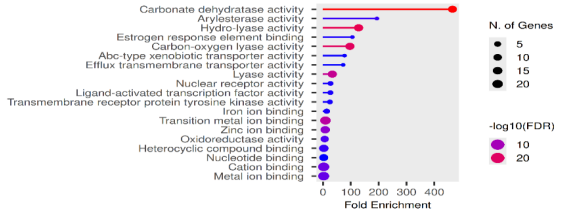


Figure 8. Bar Plot Displaying Significantly Enriched Molecular Function Terms.

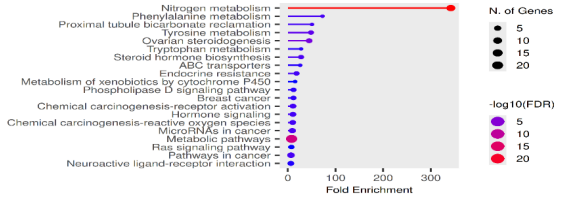


Figure 9. Bar Plot Of Significantly Enriched KEGG Pathways.

2.10. In Vitro Neuroprotective Assay

The neuroprotective activity of Sample-SN was evaluated against hydrogen peroxide (H_2O_2)-induced oxidative stress in SH-SY5Y human neuroblastoma cells using the MTT colorimetric assay [24]. Test

concentrations ranged from 12.5 to 100 µg/mL. The concentration range of 12.5–100 µg/mL was selected in accordance with previously reported in vitro neuroprotection studies involving plant extracts and phytochemicals, which commonly employ concentrations between 10 and 100 µg/mL to assess cytoprotective activity under oxidative stress conditions [25]. These concentrations allow evaluation of dose-dependent cellular responses while minimizing nonspecific cytotoxic effects [26-27].

SH-SY5Y cells were cultured under standard laboratory conditions and exposed to hydrogen peroxide (500 µM) to induce oxidative stress-mediated neuronal injury [28]. Following oxidative stress induction, cells were treated with different concentrations of Sample-SN and incubated for the specified experimental period [29]. Cell viability was subsequently determined using the MTT assay, which measures mitochondrial metabolic activity as an indicator of viable cells [30].

Briefly, MTT reagent was added to each well and incubated to allow the formation of insoluble formazan crystals by metabolically active cells. After incubation, the crystals were dissolved using a suitable solubilization solution, and absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated relative to the untreated control group and expressed as percentage viability.

A separate solvent control was not included in the study, as preliminary validation of the vehicle system demonstrated negligible effects on SH-SY5Y cell viability compared with untreated controls. This indicated minimal interference of the solvent system with cellular metabolic activity, ensuring that any observed cytoprotective effects were attributable to the bioactive constituents present in Sample-SN rather than solvent-related factors [31].

3. RESULTS

3.1. Identification And Screening Of Bioactive Compounds

A total of 10 bioactive phytoconstituents of Allium sativum were identified through literature mining and phytochemical database screening. SwissADME-based pharmacokinetic evaluation was performed to assess drug-likeness and pharmacokinetic properties. Based on Lipinski’s Rule of Five and ADME parameters, all selected compounds were considered suitable for subsequent target prediction and molecular docking studies.

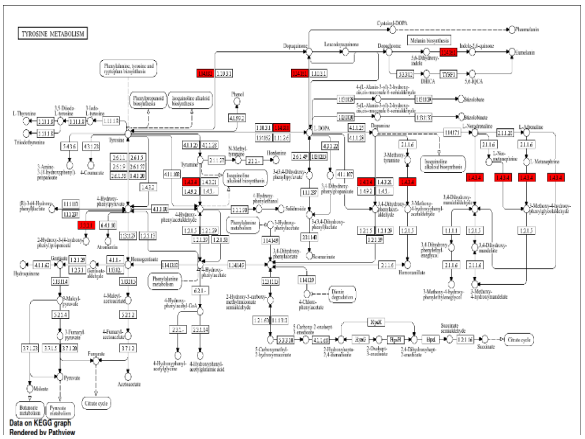


Figure 10. Mapping Of Overlapping Target Genes Onto The AMPK Signaling Pathway Using KEGG Database

3.2. Target Prediction And Identification Of Alzheimer's Disease-Associated Genes

Potential protein targets associated with the selected phytoconstituents were predicted using Swiss Target Prediction and related target prediction platforms. Alzheimer's disease-associated genes were retrieved from Gene Cards, OMIM, and Dis Ge NET databases. Following data integration and standardization through Uni Prot, overlapping targets between Allium sativum phytoconstituents and Alzheimer's disease-associated genes were identified through Venn analysis. These common targets were considered potential therapeutic mediators responsible for the anti-Alzheimer activity of Allium sativum.

3.3. Generation Of PPI Network And Detection Of Key Regulatory Genes

The overlapping targets were imported into the STRING database and used to construct a protein–protein interaction (PPI) network with a confidence score threshold of ≥0.7. The resulting network was visualized and analyzed using Cytoscape. CytoHubba analysis based on degree centrality identified ten hub genes, namely EGFR, CA1, CA4, CYP19A1, CA2, MAOA, ESR2, CA9, IGF1R, and CA12 (fig. 5).

Among the identified hub genes, EGFR exhibited the highest degree score (14), followed by CA1 (10) and CA4 (9). CYP19A1 and CA2 showed degree scores of 7, whereas MAOA, ESR2, CA9, and IGF1R each exhibited degree scores of 6. CA12 demonstrated a degree score of 5 (Table 3). These findings suggest that the identified hub genes occupy central regulatory positions within the interaction network Botanical–Bioactive–Target–Disease network and may play important roles in the therapeutic effects of Allium sativum against Alzheimer's disease (fig. 11).

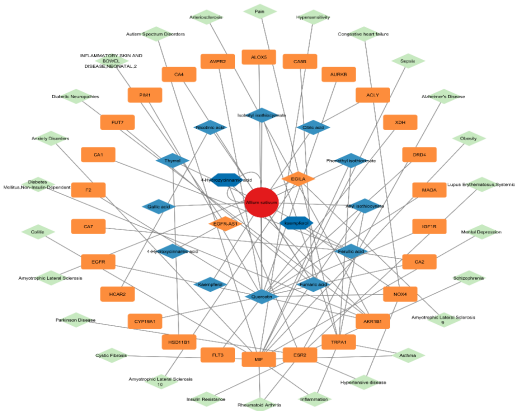


Figure 11. Botanical–Bioactive–Target–Disease Network

3.4. Molecular Docking Analysis

Molecular docking was performed to evaluate the binding affinity of ten selected phytoconstituents against ten validated Alzheimer's disease-related target proteins using CB-Dock2. Docking scores ranged from −2.0 to −5.8 kcal/mol, indicating varying degrees of protein–ligand interactions.

Among the evaluated compounds, Ajoene and Allixin demonstrated the strongest binding affinities across multiple target proteins. The highest docking score was observed for Ajoene–MAOA (PDB ID: 2Z5X) and Allixin–MAOA (PDB ID: 2Z5X), both exhibiting binding energies of −5.8 kcal/mol. Additional strong interactions included Allixin–8CO3 (−5.4 kcal/mol), Ajoene–8CO3 (−5.5 kcal/mol), Allixin–2FOY (−5.2 kcal/mol), Allixin–3D94 (−5.2 kcal/mol), and Ajoene–7XWQ (−5.1 kcal/mol) (Table 4).

Overall, Allixin exhibited the most consistent binding profile across the selected targets, with docking scores predominantly ranging between −4.5 and −5.8 kcal/mol. Ajoene also demonstrated strong target engagement and favorable interaction patterns. These findings suggest that both compounds may represent key bioactive constituents responsible for the anti-Alzheimer activity of Allium sativum.

3.5. Protein Structure Validation

To ensure structural reliability for docking analysis, all selected protein targets underwent validation using PDB-REDO, SAVES v6.0, and ProSA-web. As a representative example, MAOA (PDB ID: 2Z5X) demonstrated satisfactory structural quality following refinement (fig. 1; Table 2). ProSA-web analysis generated a Z-score of −9.95, which falls within the characteristic range of experimentally determined X-ray crystallographic protein structures of similar size. These results confirmed the suitability of the selected protein models for molecular docking studies.

3.6. Molecular Dynamics Simulation

Normal Mode Analysis (NMA) was performed using the iMODS server on the top-ranked docked complexes to evaluate structural stability and conformational flexibility. The analyzed complexes exhibited low deformability and favorable eigenvalues, indicating stable protein–ligand interactions and resistance to structural deformation. Covariance matrix and elastic network analyses further demonstrated coordinated residue movements and stable binding environments within the docking pockets (fig. 12-17). These findings supported the molecular docking results and suggested sustained target engagement of the selected Allium sativum phytoconstituents with Alzheimer's disease-associated proteins

Table 4 Docking Scores of Selected Ligands with Target Protein.

Target Protein (PDB ID)	1,3 dithiane	2-vinyl-1-3,dithiane	Ajoene	Allicin	Allixin	Allyl isothiocyanate	Allyl methyl trisulfide	Diallyl sulfide	Diallyl trisulfide	Dimethyl disulfide
1l7z	-3.5	-4	-4.3	-4.3	-4.3	-3.7	-3.5	-3.3	-3.7	-2.3

			8	8	2			9		
2foy	-3.2	-4.3	-4.9	-4.3	-5.2	-3.5	-3.4	-3.9	-3.9	-2.1
2z5x	-3.6	-4.5	-5.8	-5	-5.8	-3.7	-3.8	-3.9	-4.4	-2.3
3d94	-3.8	-4.6	-4.9	-4.4	-5.2	-3.7	-3.6	-3.9	-4.1	-2.3
3fw3	-3.0	-3.6	-4.4	-4.2	-4.5	-3.4	-3.2	-3.7	-3.3	-2.0
3s79	-3.4	-4.2	-4.8	-4.2	-4.6	-3.6	-3.5	-3.8	-4.1	-2.3
5fl6	-3.2	-4.1	-4.9	-4.5	-4.7	-3.6	-3.4	-3.3	-4	-2.2
5wb7	-3.	-3.	-4	-3	-4	-3.2	-2.	-3	-3.	-2.
	0	8	.2	.8	.3		9	.4	7	4
7xwq	-3.5	-4.3	-5.1	-4.7	-5.2	-3.9	-3.7	-3.9	-4.4	-2.3
8co3	-3.5	-4.5	-5.5	-4.7	-5.4	-3.8	-3.5	-4	-4.2	-2.4

3.7. In Vitro Neuroprotective Activity Assay

The neuroprotective activity of Sample-SN was evaluated against H_2O_2-induced oxidative stress in SH-SY5Y neuroblastoma cells using the MTT assay. Exposure of SH-SY5Y cells to hydrogen peroxide (500 µM) significantly reduced cell viability to 6.95%, confirming successful induction of oxidative stress and severe cellular damage. Treatment with Sample-SN exhibited a concentration-dependent protective effect against H_2O_2-induced neurotoxicity. The highest tested concentration (100 µg/mL) demonstrated the greatest cytoprotective activity, restoring cell viability to 59.43%. As the concentration decreased, a gradual reduction in cell viability was observed, indicating a dose-dependent neuroprotective response (Graph 1).

The observed increase in cell survival suggests that Sample-SN effectively attenuates oxidative stress-mediated neuronal damage and promotes cellular viability under neurotoxic conditions. The substantial restoration of cell viability compared to the H_2O_2-treated group highlights the potential neuroprotective properties of the phytoconstituents present in the formulation. Overall, these findings support the results obtained from the network pharmacology, molecular docking, and molecular dynamics studies, indicating that Sample-SN may exert protective effects against Alzheimer's disease-associated oxidative stress and neuronal injury".

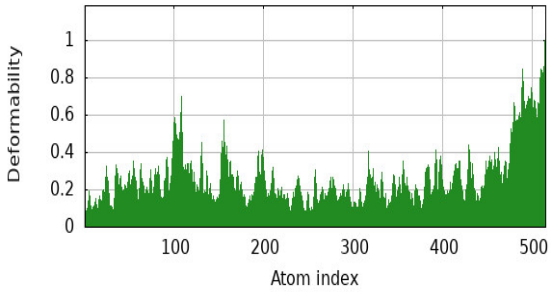


Figure 12. Deformability Plot Of The MAOA-Allixin Complex.

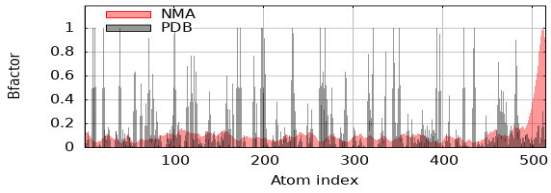


Figure 13. B-Factor Plot Representing Atomic Mobility In The Complex.

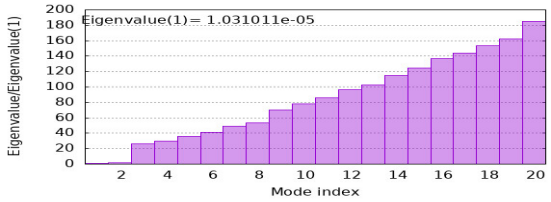


Figure 14. Eigenvalue Plot For The MAOA-Allixin Complex.

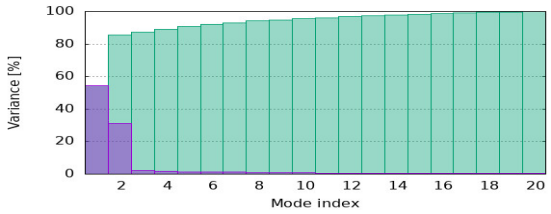


Figure 15. Variance Plot Of The MAOA-Allixin Complex.

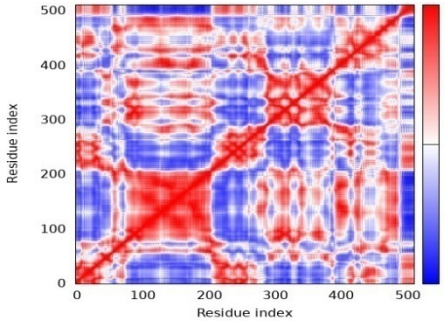


Figure 16. Covariance Matrix Of Residue Fluctuations In The Complex.

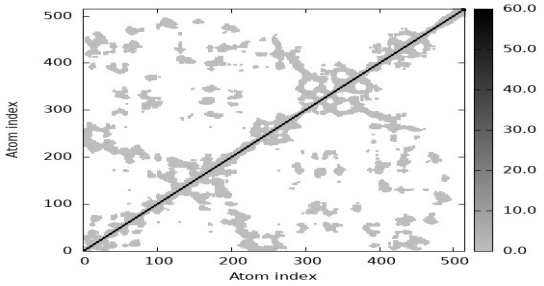


Figure 17. Elastic Network Model Showing Inter-Residue Stiffness And Connectivity.

4. Discussion

The complicated neurodegenerative ailment known as Alzheimer's disease (AD) is marked by a gradual loss of cognitive abilities, memory loss, inflammation of the brain's neurons, imbalances in neurotransmitters, buildup of amyloid-beta, and malfunctioning of the tau protein. Because of its complex nature, successful treatment approaches need the manipulation of several molecular targets instead of focusing on just one. Network pharmacology, molecular docking, and molecular dynamics modeling were all part of the integrative method used in this investigation, and in vitro neuroprotective evaluation was employed to investigate the anti-Alzheimer potential of Allium sativum.

Phytochemical screening identified 145 compounds from Allium sativum through IMPPAT database mining and literature validation. Following pharmacokinetic evaluation using Swiss ADME, 87 compounds satisfied Lipinski’s Rule of Five without violations, indicating favorable drug-likeness properties. Target prediction analysis further identified 44 high-confidence protein targets associated with these compounds. The intersection between phytoconstituent-related targets and Alzheimer’s disease-associated genes generated a set of common targets that were considered potential mediators of the therapeutic effects of Allium sativum.

Protein–protein interaction analysis revealed EGFR, CA1, CA4, CYP19A1, CA2, MAOA, ESR2, CA9, IGF1R, and CA12 as the major hub genes within the network. These proteins are involved in critical biological processes related to neuronal signaling, neurotransmitter metabolism, hormonal regulation, cellular homeostasis, and neuroprotection. Among them, EGFR emerged as the most significant hub gene, exhibiting the highest degree score. EGFR-mediated signaling has been implicated in neuronal survival and neuroinflammatory responses, suggesting its important role in Alzheimer’s disease progression. Similarly, MAOA and IGF1R occupied central positions within the network. MAOA

regulates monoamine neurotransmitter metabolism, while IGF1R contributes to neuronal growth, survival, and insulin signaling. ESR2 and CYP19A1 were also identified as important targets associated with estrogen-mediated neuroprotective mechanisms that influence cognitive function and neuronal resilience.

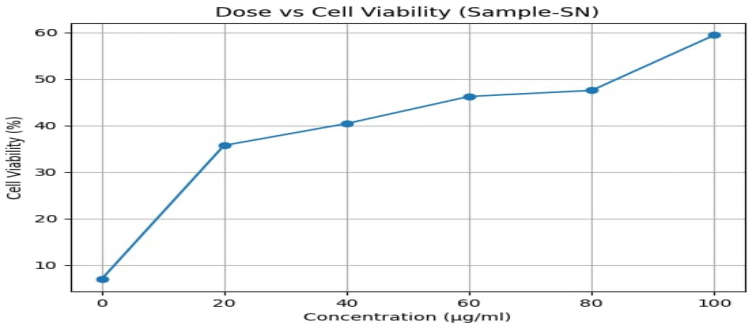
Molecular docking studies provided further evidence of the therapeutic potential of garlic-derived phytoconstituents. Among the investigated compounds, Ajoene and Allixin exhibited the strongest and most consistent binding affinities against the selected Alzheimer’s disease-associated targets. Both compounds showed the highest binding affinity toward MAOA (PDB ID: 2Z5X), with docking scores of −5.8 kcal/mol. In addition, Allixin demonstrated strong interactions with several proteins, including 8CO3, 2FOY, and 3D94, indicating broad-spectrum target engagement. Ajoene also exhibited favorable interactions across multiple proteins, supporting its potential contribution to the neuroprotective effects of Allium sativum. These findings suggest that the identified phytoconstituents may modulate pathways associated with neurotransmission, oxidative stress regulation, neuronal survival, and cellular signaling.

To validate the docking findings, molecular dynamics simulations were performed on the top-ranked protein–ligand complexes. Normal Mode Analysis revealed low deformability and favorable eigenvalues, indicating structural stability and minimal conformational changes upon ligand binding. Covariance matrix and elastic network analyses further confirmed coordinated residue motion and stable interaction environments, supporting the reliability of the docking results.

The computational findings were further validated through in vitro neuroprotective studies using H_2O_2-induced oxidative stress in SH-SY5Y neuroblastoma cells. Hydrogen peroxide exposure significantly reduced cell viability, confirming successful induction of oxidative stress-mediated neuronal injury. Treatment with Sample-SN produced a concentration-dependent protective effect, with the highest concentration (100 µg/mL) restoring cell viability to 59.43%. These results demonstrate the ability of Allium sativum constituents to protect neuronal cells against oxidative damage, a major pathological hallmark of Alzheimer’s disease.

Overall, the present study demonstrates that Allium sativum exerts anti-Alzheimer activity through the coordinated modulation of multiple molecular targets and pathways associated with neurodegeneration, neuronal survival, oxidative stress, and the metabolism of neurotransmitters. Based on these results, garlic seems to be a good natural treatment option for Alzheimer's disease.

Graph 1. Concentration Based Cell Viability.



5. CONCLUSION

The in vitro neuroprotective assessment, molecular docking, network pharmacology, and molecular dynamics modeling were all part of the integrated method that this research used to evaluate Allium sativum's anti-Alzheimer potential. The IMPPAT database and literature sources were used to identify 145 phytoconstituents in total, of which 87 compounds satisfied drug-likeness criteria based on SwissADME analysis. Further target prediction identified 44 high-confidence protein targets, which were subsequently analyzed against Alzheimer’s disease-associated genes to elucidate potential therapeutic mechanisms.

Protein–protein interaction network analysis identified key hub genes, including EGFR, CA1, CA4, CYP19A1, CA2, MAOA, ESR2, CA9, IGF1R, and CA12, which were associated with neuronal signaling, neurotransmitter metabolism, hormonal regulation, and neuroprotective pathways relevant to Alzheimer’s disease. Molecular docking studies demonstrated favorable interactions between Allium sativum phytoconstituents and the selected target proteins. Among the evaluated compounds, Ajoene and Allixin exhibited the strongest binding affinities, with docking scores reaching −5.8 kcal/mol against MAOA (PDB ID: 2Z5X), suggesting their potential role as key bioactive constituents. These interactions were further supported by molecular dynamics simulation, which confirmed the structural stability and favorable conformational behavior of the protein–ligand complexes.

The MTT test, which is used for in vitro validation, showed that Sample-SN significantly protected SH-SY5Y neuroblastoma cells from oxidative damage caused by H_2O_2. Restoring cell viability to 59.43% at the highest dose (100 µg/mL) demonstrated significant protection against neuronal damage caused by oxidative stress. The computational predictions and these experimental results provided support for the multi-target mechanism of action that was postulated.

Taken as a whole, the results show that Allium sativum might be a useful natural multi-target drug in treating Alzheimer's disease. There is scientific evidence that Allium sativum may be a useful neuroprotect

ive intervention due to its ability to modulate important molecular targets associated with neurodegeneration, oxidative stress, and neuronal survival. We need further clinical trials and in vivo research to confirm its safety, effectiveness, and therapeutic potential

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